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(54) **METHOD FOR ADJUSTING A GRADIENT DELAY VOLUME**

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(2013.01)

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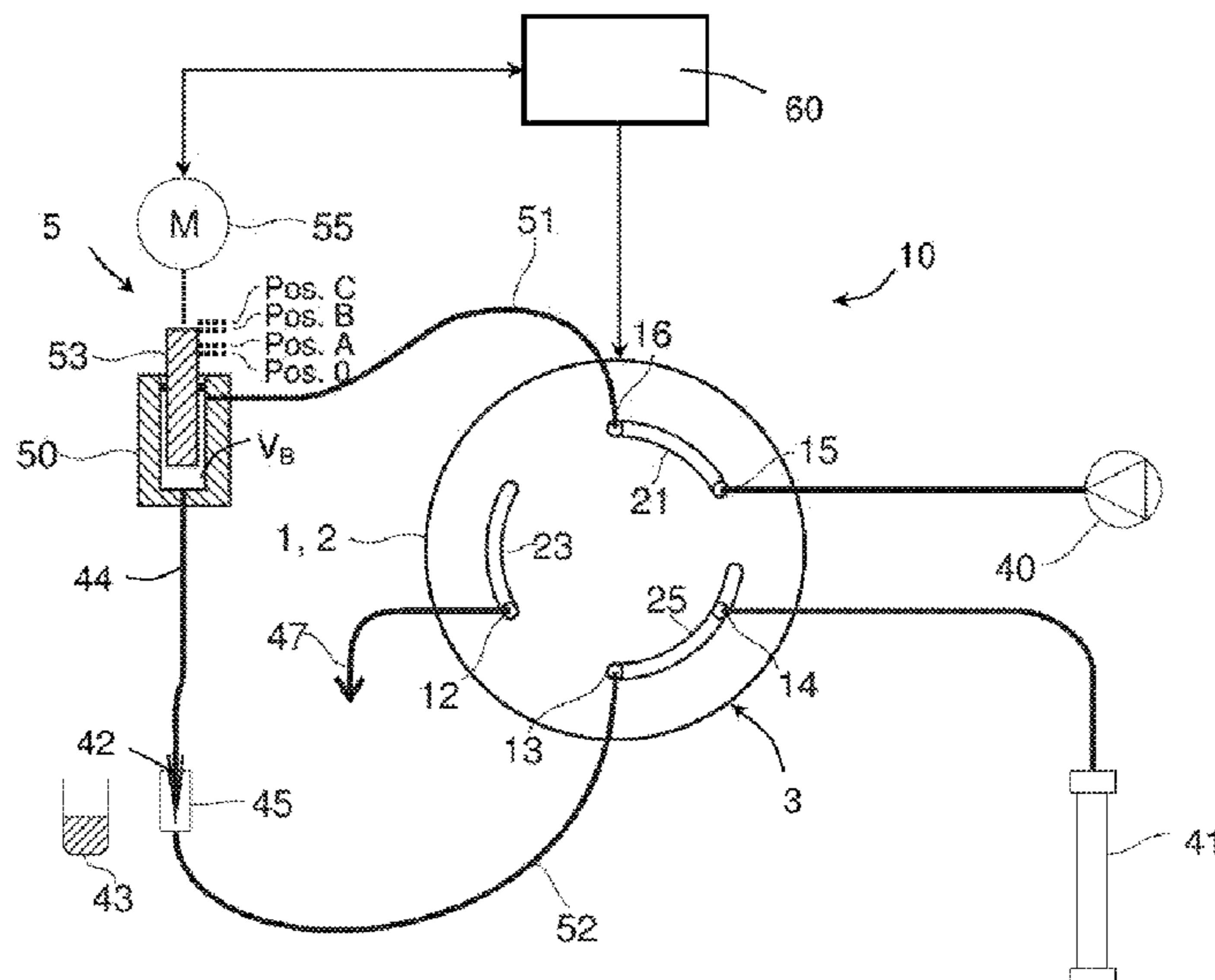
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*Primary Examiner* — Pamela H Weiss

(57) **ABSTRACT**

The invention relates to a method for setting a gradient delay  
volume GDV of a liquid chromatography system for a  
chromatography run in liquid chromatography, in particular  
a high-performance liquid chromatography system, in which  
a desired value  $GDV_{target}$  of a gradient delay volume of the  
liquid chromatography system is ascertained or predefined  
and, if the value  $GDV_{target}$  deviates from a specific fixed  
value  $GDV_{actual}$  of a liquid chromatography system, this  
value  $GDV_{target}$  is set in a range  $0 \leq \Delta GDV = GDV_{target} -$   
 $GDV_{actual} \leq V_{max}$  of a volume of a volume adjustment device  
5. Furthermore, the invention relates to an automatic sam-  
pler for carrying out such a method.

**10 Claims, 7 Drawing Sheets**



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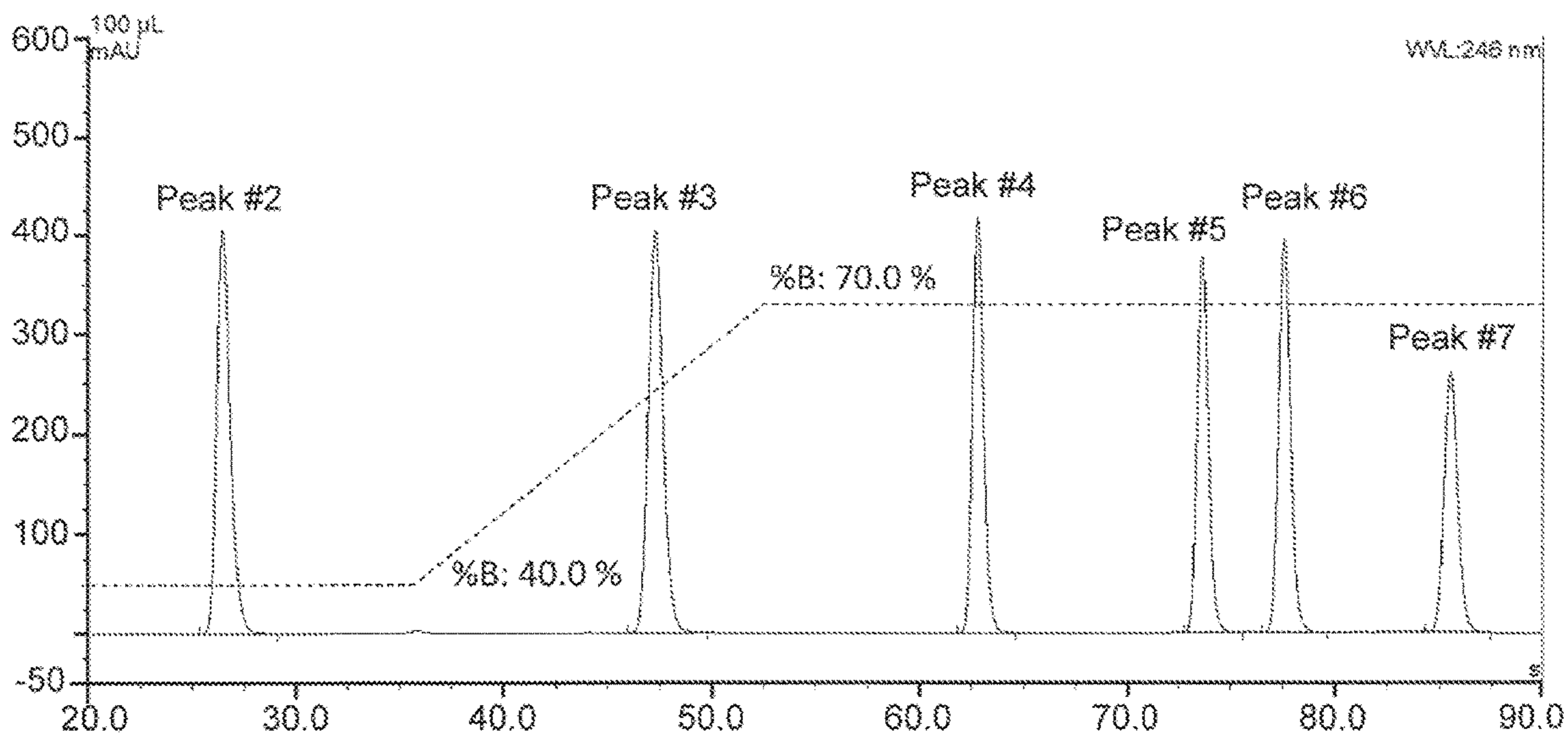
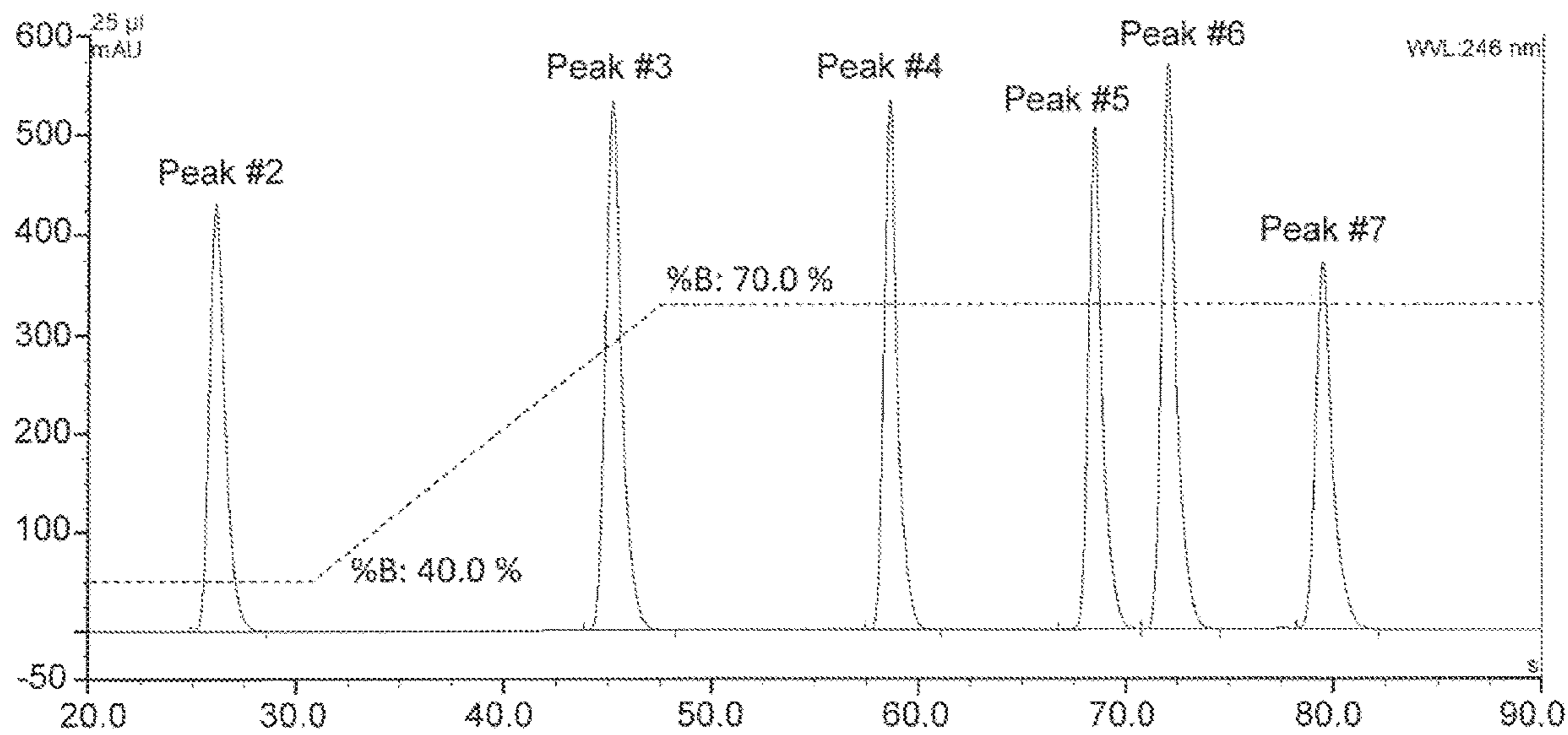


Fig. 1

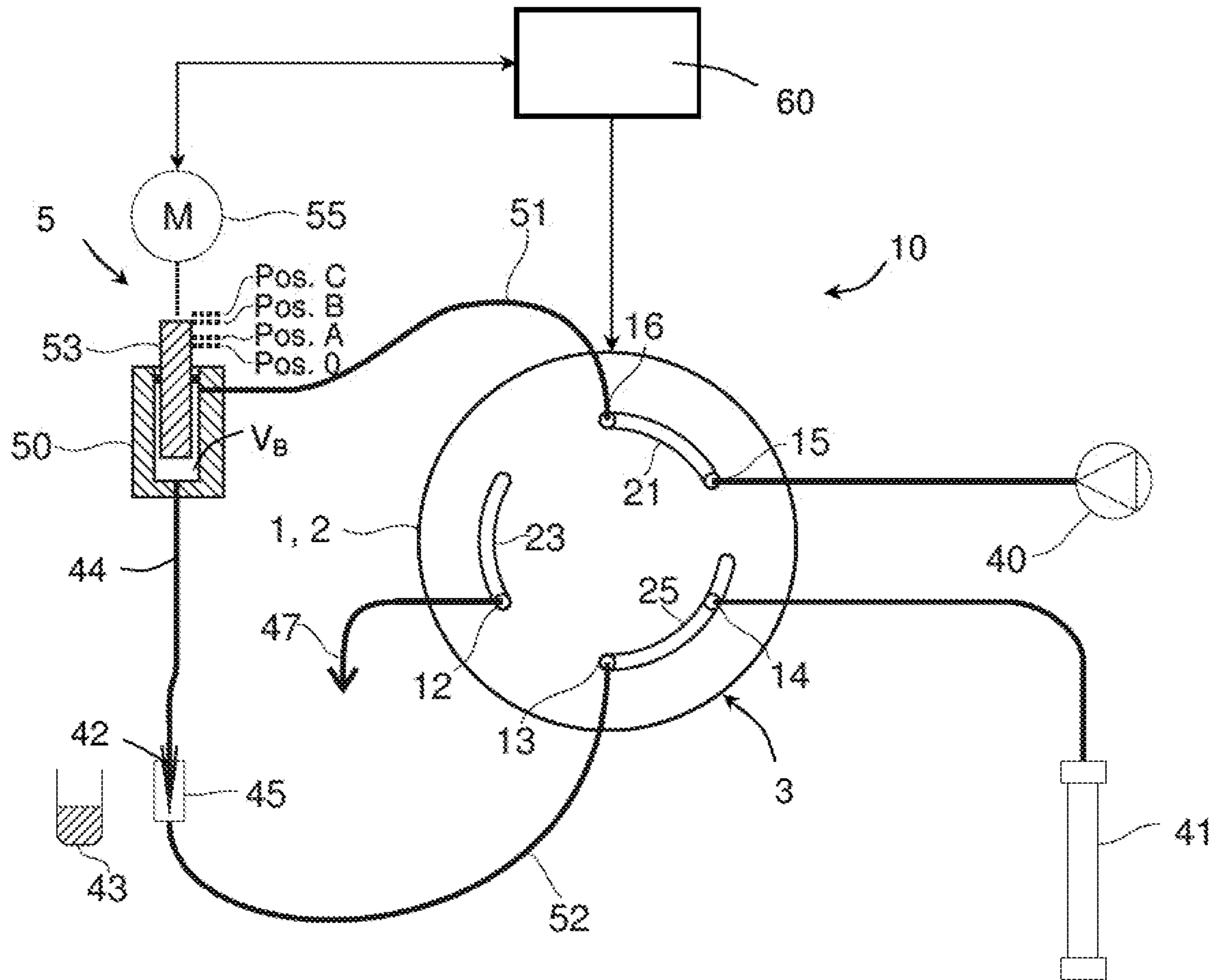


Fig. 2

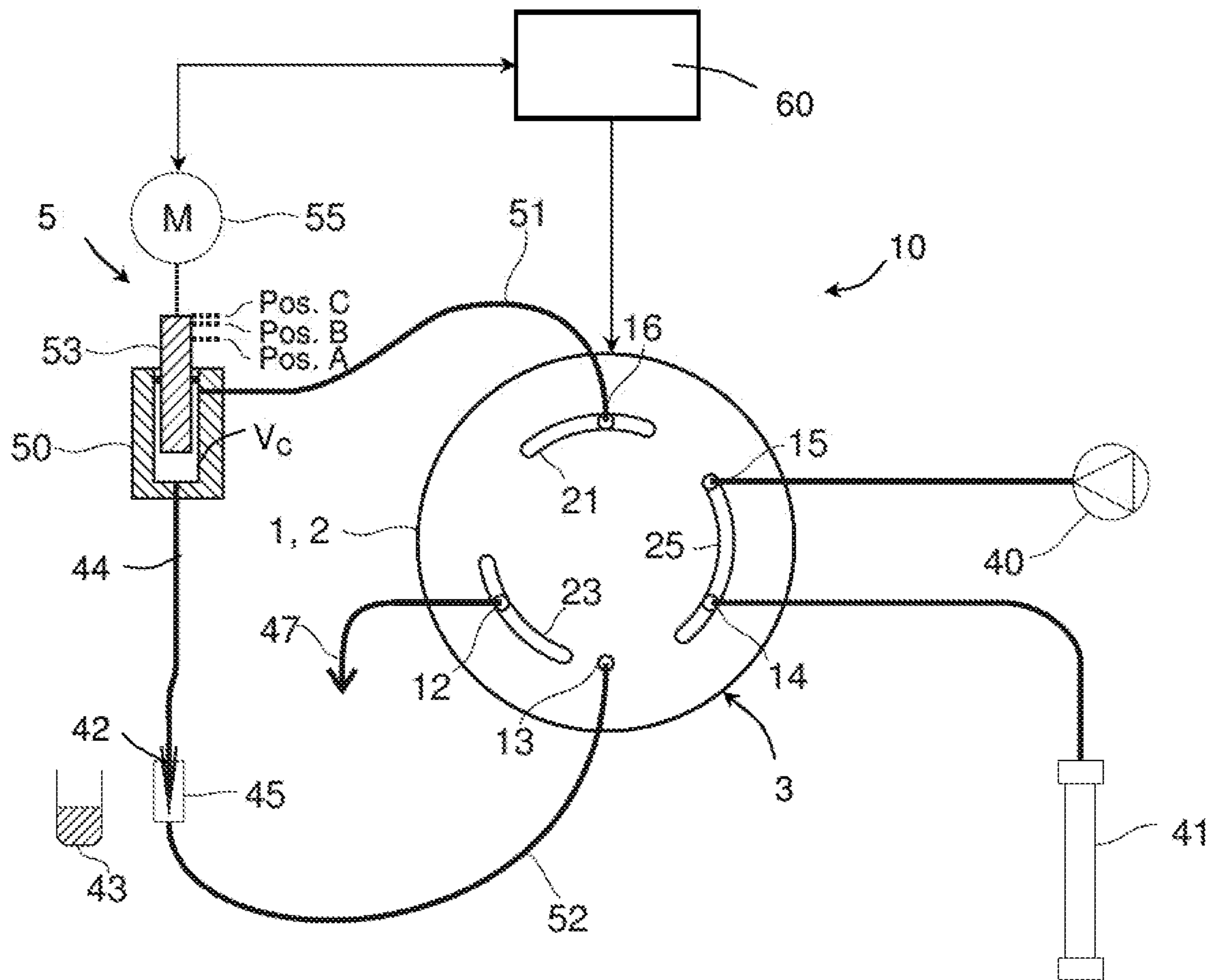


Fig. 3

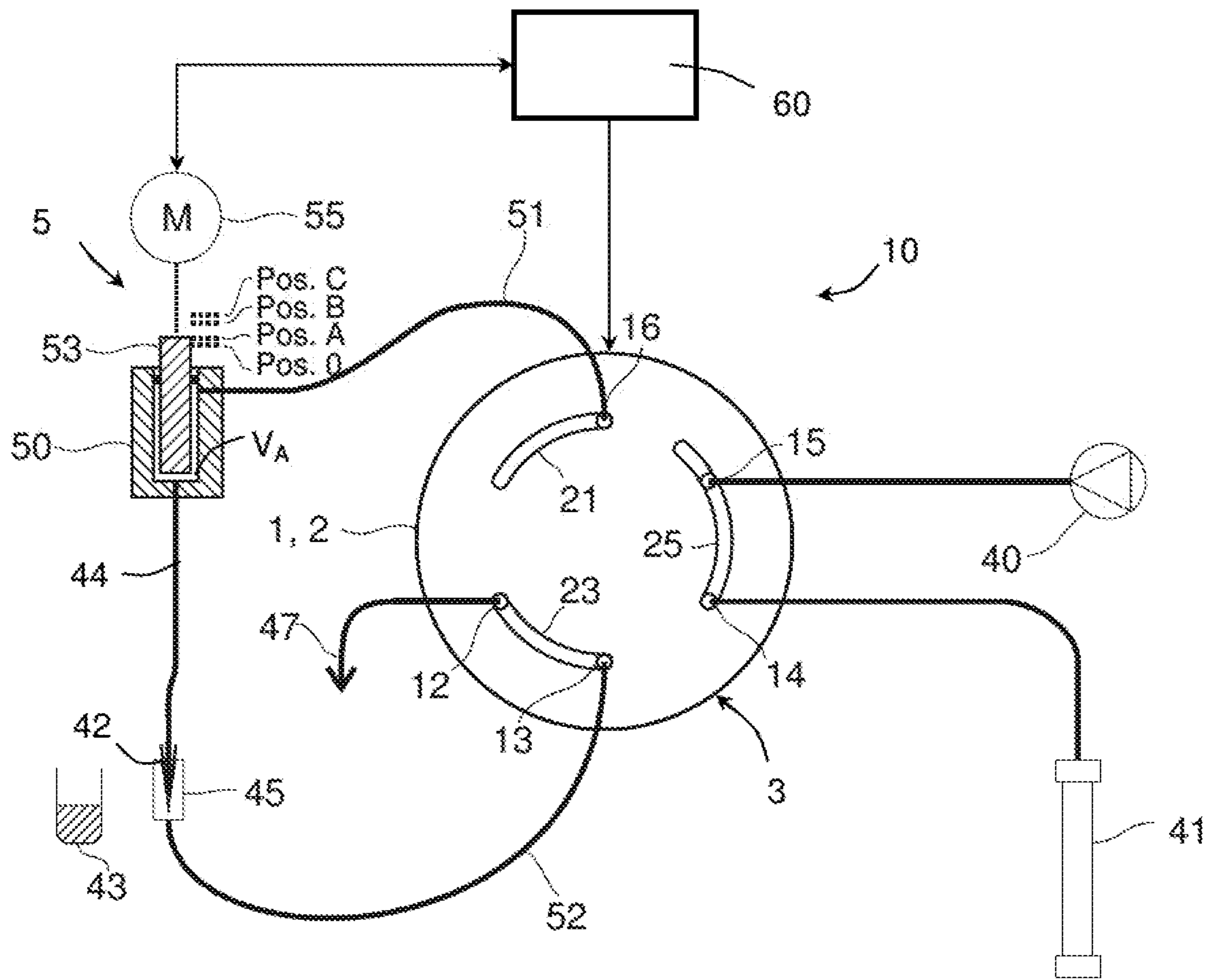


Fig. 4

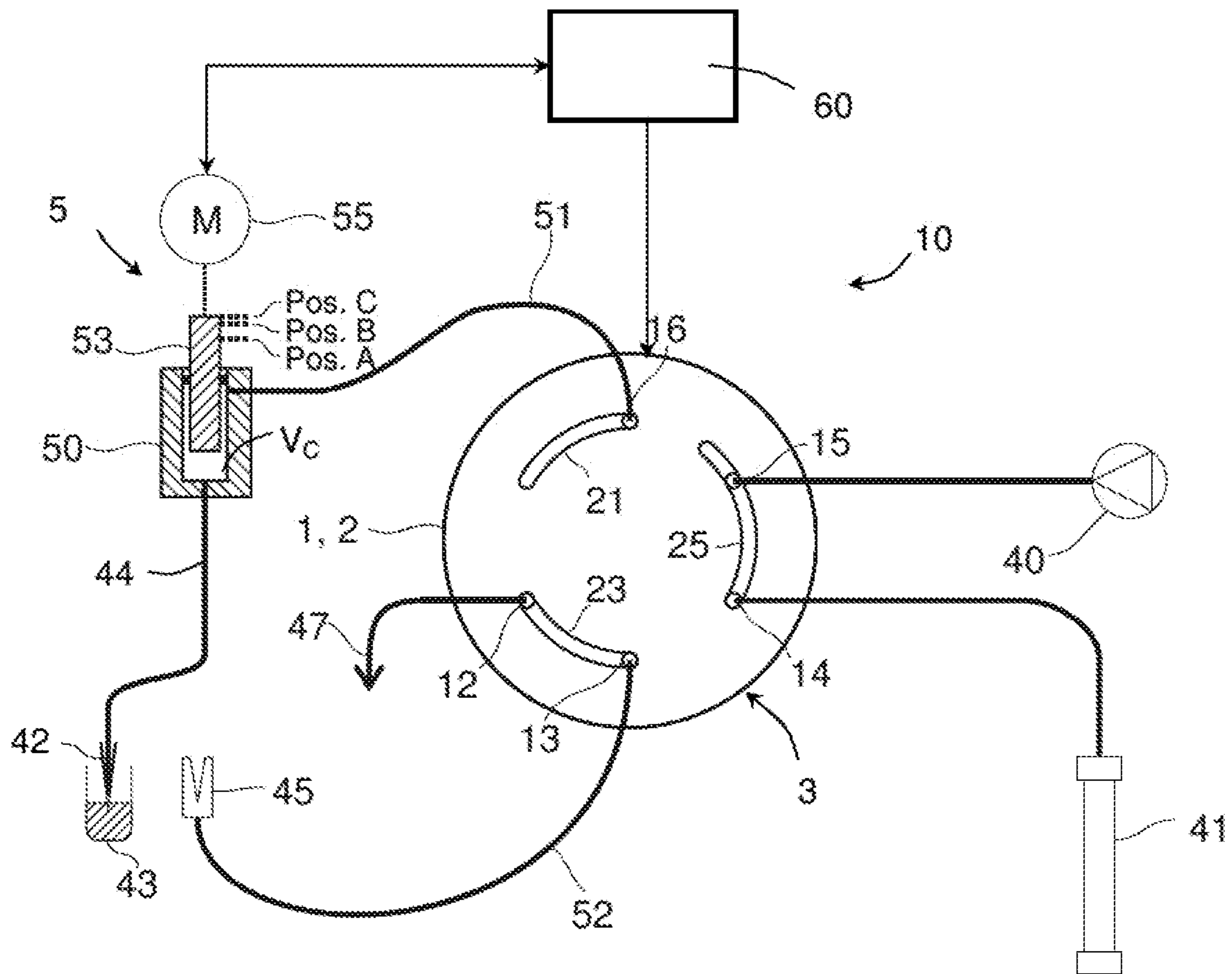


Fig. 5

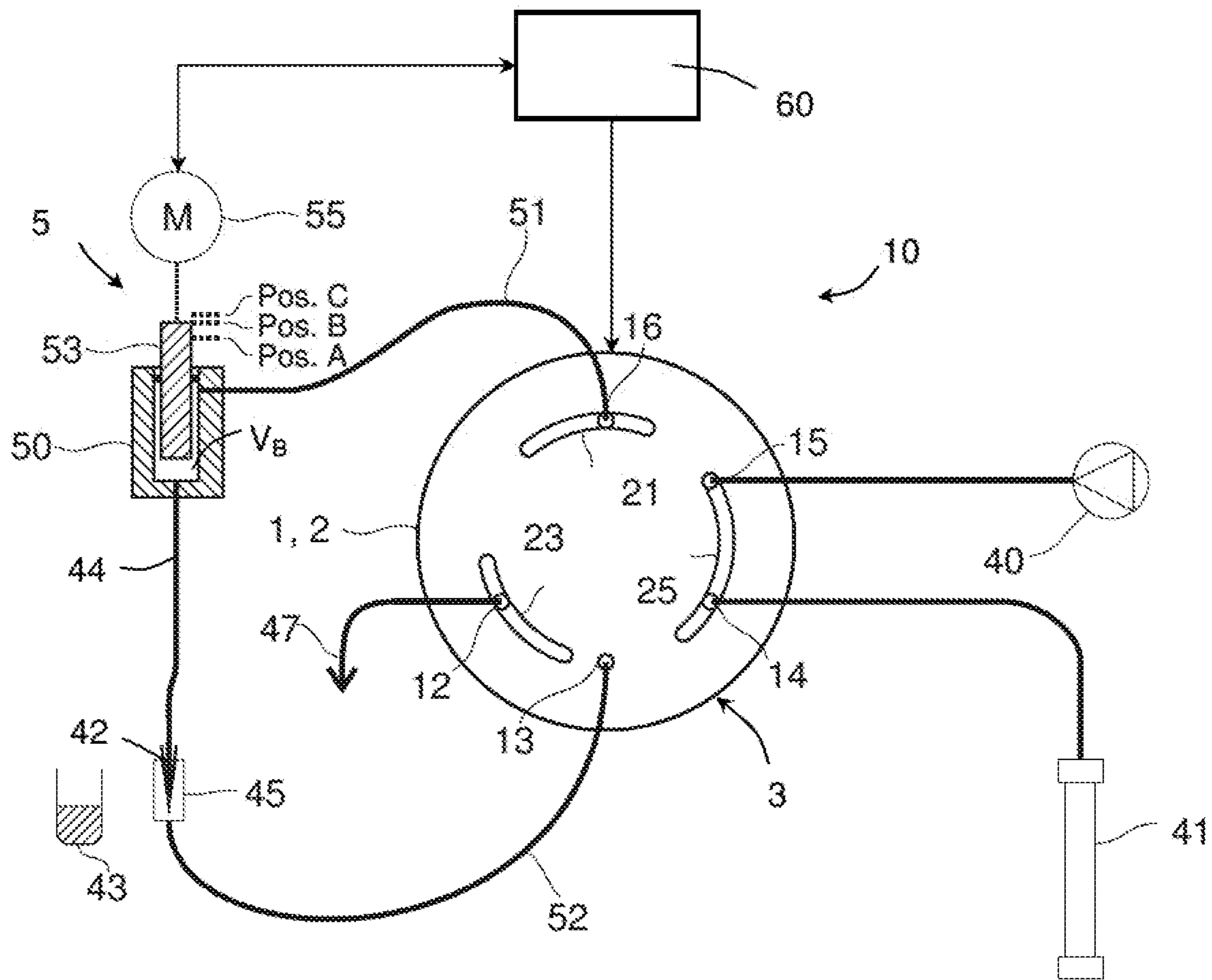


Fig. 6



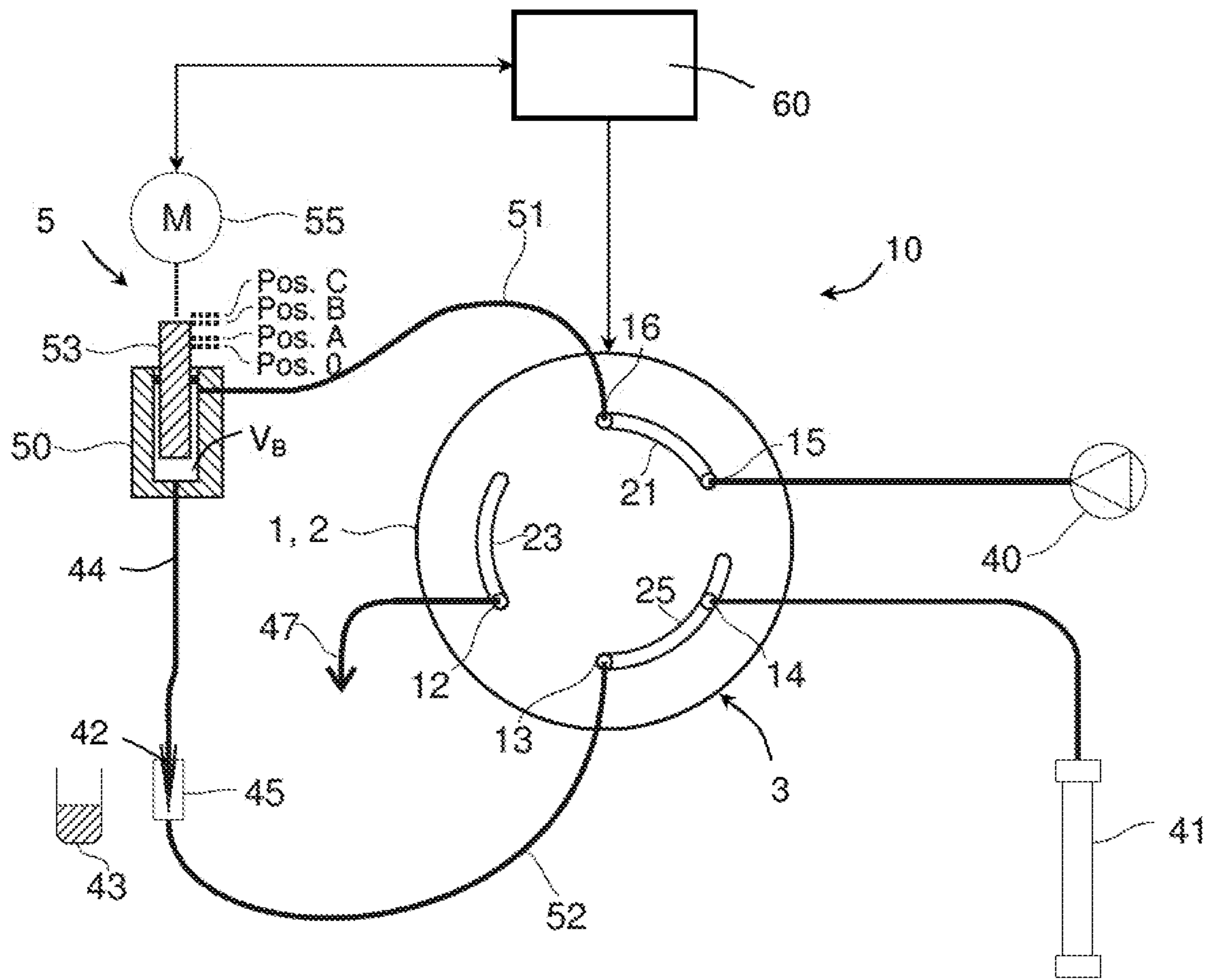


Fig. 7

## METHOD FOR ADJUSTING A GRADIENT DELAY VOLUME

### CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation under 35 U.S.C. § 120 and claims the priority benefit of co-pending U.S. patent application Ser. No. 14/661,793, filed Mar. 18, 2015, which claims the priority benefit under 35 U.S.C. § 119 to German Patent Application No. 10 2014 103 766.9, by Hermann Hochgraeber and Thomas Wachinger for “A method of adjusting a gradient delay volume” filed on Mar. 19, 2014, the disclosure of which is incorporated herein by reference.

### FIELD OF THE INVENTION

The present invention is in the field of liquid chromatography, in particular high-performance liquid chromatography (HPLC).

### BACKGROUND

Liquid chromatography (in particular HPLC) is used for the purpose of separating liquid samples into their components by means of a chromatography column. In this case, the separating power of the (separating) column is dependent, inter alio, on its length and on the particle size of the packing material. For the best possible separation, columns having a sufficient length and a small particle size are required. Such columns have a high flow resistance and therefore require substantially higher pressures for operation than conventional columns.

Furthermore, a sufficiently rapid separation is desirable, to enable a high sample throughput. This requires a high flow speed in the column, whereby the counterpressure in the column also increases.

One possibility for accelerating the separation or increasing the separating power is to change the solvent composition over the duration of the separation (referred to hereafter as the gradient, i.e., the degree of the change of the elution force or the proportion of a solvent with time is referred to as the gradient or solvent gradient).

Two different technical implementations for achieving a gradient have prevailed due to the different requirements for the separation. These are so-called high-pressure gradient forming (HPG) and low-pressure gradient forming (LPG). High-pressure gradient forming operates by means of two independent pumps, which are connected to one another via a T-part on the high-pressure side of the system. The gradient is generated by way of the change of the flow rates at the two pumps. Low-pressure gradient forming only requires one pump having proportioning valve unit connected upstream. During the aspirating cycle of the pump, the various solvents are drawn successively into the pump by opening and closing the valves (for example, solenoid valves) in the proportioning valve unit. The gradient is formed due to the variations in the opening times for the various solvents. To smooth out composition fluctuations in the solvent mixture (mixture irregularity), a mixer is connected downstream from the pump as a standard feature.

In the various designs, there are different so-called gradient delay volumes (GDV—or also dwell volume). The gradient delay volume or mixing volume is dimensioned, on the one hand, by way of the holding capacity of all interconnected components from the mixing point up to the entry of the column. In the case of LPG, the GDV—depending on

the implementation of the switching valve and the connecting ports which are switched through—is formed, for example, by the volumes of the following components (or a part thereof): pump head, mixer, connecting capillaries, sample loop, switching valve, metering device. In the case of HPG, the GDV—depending on the implementation of the switching valve and the connecting ports which are switched through—is formed, for example, by the volumes of the following components (or a part thereof): T-part, mixer, connecting capillaries, sample loop, switching valve, metering device. On the other hand, the washing-out volume must also be considered, which results due to the flow properties of the components.

If a chromatographic method was developed on a specific HPLC system, it usually cannot be transferred to another system without problems. The reproduction of a published method is also just as difficult. One cause of this is the different GDVs, which are accompanied by a shift and/or spreading of the retention times.

Furthermore, the mixture irregularity is also dependent on the GDV. The higher the GDV, the better the various solvents are mixed and the less the mixture irregularity.

The requirements for the GDV and the remaining mixture irregularity are also strongly dependent on the application and the detector type used. Non-optical detectors, such as MS, ELSD, or CAD, are typically insensitive to mixture irregularities, since these do not generate signal variations due to differing detection sensitivities to the individual solvent components. A mixer having small GDV with moderate mixer performance would therefore be acceptable in such a case. The situation appears very different, however, upon the use of a UV detector, above all at low wavelengths. Extremely small variations of the solvent composition have effects here in visible variations in the baseline. This makes it more difficult to determine the material concentration from the detector signal. A mixer having the highest possible mixing efficiency with correspondingly greater GDV would be advantageous in this case.

An unnecessarily large GDV is also advantageous, since the analysis, washing, and equilibration phases would also be increased unnecessarily.

If a method is transferred to another HPLC system, which has deviating GDVs, an offset time ( $tg_{dv} = V_{gdv}/\text{flow rate}$ ) could be calculated and the gradient forming could be started delayed or early accordingly.

However, problems with the irregularity cannot be completely remedied in this way. For this purpose, the mixer is normally manually replaced with another mixer. Of course, automation of such a replacement by means of a switching valve would also be conceivable. This solution would be cumbersome because of the size of the mixers and additionally costly, however.

### SUMMARY

The present invention is therefore based on the object of providing a method and a system or an automatic sampler for carrying out this method, which enable an adaptation of the GDV or mixing volume in a cost-effective and structurally simple manner.

According to the invention, in a chromatography system between mixing point (gradient occurrence) and entry of a separating column, a volume adjustment device is provided, which enables simple, automated, and cost-effective change of the GDV or mixing volume. Such a volume adjustment device offers a preferably continuously settable volume, for example, via a control device, through which a solvent

(gradient), which changes in composition, flows through during a chromatography run and correspondingly counts for the GDV.

Depending on the implementation of the chromatography system, components are located in the path—in or through which a gradient is navigated—for example:

in LPG—depending on the implementation of the switching valve and the connecting ports which are switched through: pump head, mixer, connecting capillaries, sample loop, switching valve, metering device, or parts thereof and;

in HPG—depending on the implementation of the switching valve and the connecting ports which are switched through: T-part, mixer, connecting capillaries, sample loop, switching valve, metering device, or parts thereof.

The holding capacity or the internal volume through which a gradient flows of the components located in each case in this path therefore result in a specific GDV for a respective system.

By way of the method according to the invention and by way of the liquid chromatography system according to the invention it is now possible to provide a desired GDV—deviating from a system-specific (fixed) GDV. Thus, a predefined GDV, which is ascertained during a method development or is known, for example, of another system can be set, without components (mixers, etc.) having to be changed, added, replaced, or switched for this purpose on the present system. The change of the GDV is performed in this case in a range between the maximum and minimum adjustable volumes, for example, 0  $\mu\text{L}$  to 1000  $\mu\text{L}$ , in particular 0  $\mu\text{L}$  to 500  $\mu\text{L}$ , preferably 0  $\mu\text{L}$  to 120  $\mu\text{L}$ , of the volume adjustment device, preferably by means of an electronic control unit.

In a further embodiment of the invention, the desired value is continuously adjustable by corresponding adjustment of the volume adjustment device. Of course, it is also conceivable to provide discrete steps within the variable range of the GDV, however.

In a preferred embodiment of the invention, a metering device which is already provided in the system, in particular in the automatic sampler, for taking samples and/or injecting samples, is used as the volume adjustment device. The additional use of an already provided component enables a structural and cost-reducing savings potential.

In an arbitrary embodiment of the invention, the desired GDV can be preset before a chromatography run (in which a gradient is navigated), for example, before taking a sample or before injecting a sample, or can be set during an injection or during a chromatography run.

In an arrangement (or with an automatic sampler), in which the metering device or the holding capacity thereof counts for the GDV, in this way, the GDV can be changed by a preset holding capacity (greater than 0 or deviating from a system-specific starting setting for the removal) before taking the sample, since this preset volume, which deviates from the standard position, counts for the GDV after switching over the paths (removal—injection).

Of course, however, it is also conceivable to set or vary the GDV during and/or after a sample injection. Preferably, a pump device (also already provided in a chromatography system), in particular a solvent pump, can be activated in opposition accordingly in this case, so that the resulting volume stream or the flow rate and/or the pressure in the path to the separating column, in particular before the entry thereof, is kept essentially constant (i.e., with a deviation from the existing flow rate or the existing pressure of less than 20%, for example, less than 10%, in particular less than

5%, preferably less than 1%) and damage to the separating column can be avoided and the reproducibility of the analysis can be ensured.

If the change of the GDV is performed, viewed in the flow direction to the column, after an introduced sample or a so-called sample plug (i.e., in the region between mixing point or occurrence of the gradient and injection point), the run time of the gradient also changes in relation to the position of the sample plug. Correspondingly, during the analysis, for example, during the increase of the GDV, chronological spreading or stretching of the result occurs (or of the components of the sample detected as peaks over time). However, if the change of the GDV or  $\Delta\text{GDV}$  takes place, viewed in the flow direction of the column, before an introduced sample or a so-called sample plug (i.e., in the region between the injection point and entry of the separating column), the run time of the gradient remains unchanged in relation to the sample plug (no spreading), however, the result (or the detected peaks over time) occurs with a delay with the factor  $\Delta\text{GDV}/\text{flow rate}$ .

If an already provided metering device is used as the volume adjustment device and the gradient flows through the metering device, a volume which is additionally set or varied in addition to a predefined sample volume counts for the GDV. In this embodiment of the invention, it is possible to set the desired GDV in a range from 0 or minimum of the metering device up to its maximum minus the predefined sample volume.

In a further embodiment of the invention, the smallest volume of the volume adjustment device can be set at the end of a chromatography run according to this, to shorten the time for washing out to a minimum.

In addition to the above-mentioned advantages (setting the GDV continuously and without manual intervention, cost-effective usage of an existing component for this purpose, possible saving of a mixer or assistance of the mixer), an undesired, excessively large GDV in the system can also be avoided or remedied according to the invention, by simply changing the GDV or the mixing volume. In any case, it is possible according to the invention, in contrast to conventional methods and systems, to meet all application-specific requirements for GDV and mixing performance, without replacing the corresponding components.

Although the GDV or mixing volume during a chromatography run is constant in conventional methods, it is furthermore also conceivable according to the invention to vary the GDV over the time of the analysis. If one block in HPG, for example, requires very little solvent in contrast to the other block, a higher GDV is then required to suppress the mixture irregularity. At another point in time, when both blocks require approximately equal amounts, a lesser GDV can be sufficient.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be explained in greater detail hereafter on the basis of an exemplary embodiment illustrated in the drawing.

FIG. 1 shows two diagrams to illustrate the effect of two different GDVs during a chromatography run on the detected result.

FIG. 2 shows a schematic illustration of an HPLC system having an automatic sampler according to the invention, to which a chromatography column is connected, wherein the injection valve is located in the INJECT position and an equilibration phase takes place in the illustrated state.

## 5

FIG. 3 shows the HPLC system in FIG. 2, wherein the injection valve was switched from the INJECT position into the PRESSURE EQUALIZATION position and the sample loop was switched out of the analytical path.

FIG. 4 shows the HPLC system in FIG. 3, wherein the injection valve was switched into the WASTE position.

FIG. 5 shows the HPLC system in FIG. 4, wherein the sample needle was moved into the sample container to take a sample and subsequently the plunger of the syringe was moved into the end position (position C) to aspirate the sample volume.

FIG. 6 shows the HPLC system according to FIG. 5, wherein the injection valve was switched from the LOAD position into the PRESSURE EQUALIZATION position and subsequently the plunger was moved into the position B for pressure equalization (pressure increase) in the sample loop.

FIG. 7 shows the HPLC system in FIG. 6, wherein the injection valve was switched from the PRESSURE EQUALIZATION position into the INJECT position.

## DETAILED DESCRIPTION OF EMBODIMENTS

The diagrams illustrated in FIG. 1 show how a change of the GDV effects an analysis over time during a (sample) injection at the point in time 0 minutes. A solvent composition over time is shown as a dashed line in the diagrams. The solvent composition changes (transitions in the form of inflection points) from a constant (lower or front horizontal section) lower limit (acetonitrile 40%) via a varying (from 40% to 70% acetonitrile) composition or gradient (linear, rising, middle region) up to a constant (horizontal upper or rear section) upper limit (acetonitrile 70%). As the gradient, for example, a gradient having an increase of a solvent proportion B, for example, acetonitrile ACN, from 40%-70% in 16 seconds (and corresponding reduction of the solvent proportion A or the remaining solvent proportions from 60%-30%) is used. In the upper diagram (case A), the preset pump volume of the volume adjustment device is  $V_A=25 \mu\text{L}$ , i.e., the GDV is increased by  $25 \mu\text{L}$ , while in contrast in the lower diagram (case B), the adjusted pump volume is  $V_A=100 \mu\text{L}$ , i.e., the GDV is increased by  $100 \mu\text{L}$ .

Since the increase of the GDV ( $\Delta\text{GDV}=75 \mu\text{L}=100 \mu\text{L}-25 \mu\text{L}$ ) from case A to case B by the volume adjustment device, in particular a metering device 5 in the exemplary embodiment, viewed in the flow direction toward the (separating) column 41, with respect to the position of an introduced sample plug, is performed after the sample plug (i.e., following the rear separating surface between sample plug and solvent or between mixing point and tip of the sample needle 42), the incidence and therefore effect of the gradient or of a respective concentration with respect to the sample is shifted to the entry of the separating column 41, without the point in time of the incidence of the sample itself being delayed. As a result of the time shift or delay of the incidence of the respective concentration at the entry of the column 41, however, the eluting effect of the gradient on components to be eluted of the sample is shifted or delayed. As a result thereof, chronological spreading or stretching of the (sample) components detected as peaks occurs, which increases with components which are more difficult to elute (in the direction of the time axis).

As is apparent in FIG. 1 (lower diagram) as a detail of an analysis result (with illustrated peak 2 to peak 7 in mAU=milli absorption unit), the individual peaks occur with greater delay with increasing time in relation to their occurrence in the upper diagram, the later the corresponding

## 6

components are eluted (due to a higher concentration). The points in time of the occurrence of the peaks increase accordingly as shown hereafter.

Peak 2 from 26.090 s to 26.450 s	delay $\Delta t = 0.360 \text{ s}$
Peak 3 from 45.150 s to 47.250 s	delay $\Delta t = 2.100 \text{ s}$
Peak 4 from 58.520 s to 62.780 s	delay $\Delta t = 4.260 \text{ s}$
Peak 5 from 68.390 s to 73.630 s	delay $\Delta t = 5.240 \text{ s}$
Peak 6 from 71.960 s to 77.560 s	delay $\Delta t = 5.600 \text{ s}$
Peak 7 from 79.430 s to 85.520 s	delay $\Delta t = 6.090 \text{ s}$

The minimal delay at peak 2 is less than a measurement tolerance in this case, so that the occurrence thereof in case A to case B can be considered to be simultaneous or without delay. The reason for this is that the isocratic part of the solvent was already eluted at this peak 2, without a gradient already acting at this point in time.

In contrast, the amplitude or the maximum of the absorption can remain the same or be reduced, as is apparent from FIG. 1.

As a result of the GDV change  $\Delta\text{GDV}$ , the transitions or inflection points shift to the right between horizontal (isocratic) and the linear slope (gradient) on the time axis in the case of the transition horizontal to slope from 31 seconds to 36 seconds and in the case of the transition slope to horizontal from 47 seconds to 52 seconds in the comparison of the upper diagram to the lower diagram of FIG. 1. This delay of approximately 5 seconds results due to the higher GDV (lower diagram) by  $\Delta\text{GDV}=75 \mu\text{L}$  and the flow rate of  $0.95 \text{ mL/min} (=15.83 \mu\text{L/s})$ .

In contrast, if the increase of the GDV, viewed in the flow direction toward the column 41, takes place in position before the sample plug (i.e., between needle seat or injection port 45 and entry of the separating column 41 or before the front separating surface between sample plug and solvent), the detected result or peak of the components of the sample is delayed in time or shifted to the right in the diagram by the changed runtime without stretching.

FIG. 2 to FIG. 7 show, in a schematic illustration, an HPLC system having an automatic sampler 10 operating according to the split loop principle, which has a metering device 5, an injection valve 3, and a high-pressure pump 40. In addition, the automatic sampler 10 has a sample loop, which consists of a first connecting part 51, a second connecting part 52, 44, and a pump volume V (depending on the plunger position, minimum,  $V_A$ ,  $V_B$ , or  $V_C$  up to maximum  $V_{max}$  of, for example,  $120 \mu\text{L}$ ). This can be a pressure-resistant line having a small diameter, for example, in the form of glass capillaries or stainless steel capillaries. The connecting part 51 is connected to a first sample loop port 16 of the injection valve 3 and to the sample delivery unit or the pump volume V thereof. The second connecting part, which consists of an aspirating part 44 and a supply part 52, is implemented as separable. For this purpose, the supply part 52 opens into an injection port 45, which is connected via the supply part 52 to a second sample loop port 13 of the injection valve 3. The aspirating part 44, which is connected at one end to the pump volume V of the metering device 5, has a sample needle 42 at the other end, using which the aspirating part 44 can be connected to the injection port 45.

However, the sample needle 42 can also be moved toward a sample container 43 and can aspirate a defined sample volume therefrom—in the manner described hereafter—into the aspirating part 44. Furthermore, the sample needle 42 can also be moved toward a container for a flushing fluid (not shown), to take flushing liquid therefrom for a flushing

operation, using which the sample loops **51**, **52**, **44**, the pump volume  $V$ , and optionally also the ports and grooves or channels of the injection valve can be cleaned. However, due to the special topology of the illustrated split loop principle, flushing of the sample loops **51**, **52**, **44** and of the sample delivery unit **5** is normally not necessary, since the latter are flushed through with solvent, which is delivered by the pump **40**, in any case during an injection operation. However, the outer side of the sample needle **42** can be cleaned by the immersion in a container having cleaning or flushing liquid. Alternatively, the needle **42** can also be moved toward a washing and/or waste port (not shown in the drawing), to be cleaned and/or to discard excess solvent.

The metering device **5** comprises a syringe **50** in the illustrated embodiment, in which a plunger **53** is guided in a pressure-tight and displaceable manner. The plunger **53** is driven by means of a drive **55**, for example, a stepping motor. The drive **55** is activated by a control unit **60**. The control unit **60** also controls the switching operations of the injection valve **3** which has an activatable drive (not shown).

A waste port **12** of the injection valve is connected to a waste line **47**, from which fluid can be discharged into a waste reservoir (not shown).

The high-pressure pump **40** is connected to a high-pressure port **15** of the injection valve. A chromatography column **41** is connectable to the further high-pressure port **14**. The high-pressure pump **40** can be integrated as a component into the automatic sampler, however, it can also be provided in another unit or a separate pump unit.

The injection valve **3** consists of a stator **1** and a rotor **2**. The stator **1** has the two high-pressure ports **14**, **15**, the two sample loop ports **13**, **16**, and the waste port **12**. Of course, instead of the illustrated injection valve, injection valves having more than 5 ports are also conceivable for implementing the invention. Via these ports, the injection valve **3** is connected via the above-described connecting lines, which can be implemented as capillary connections, to the other functional elements of the HPLC system. The high-pressure screw connections required for this purpose are not shown for the sake of comprehensibility in FIG. **1**. For reasons of simplicity, the injection valve is shown in the interface between stator **1** and rotor **2**, wherein both the embodiment of the end face of the stator **1** and also the embodiment of the end face of the rotor **2** are shown to make it easier to understand the mode of operation. Inside the injection valve **3**, the ports are implemented as boreholes, which lead to the other side of the stator **1**. The rotor **2** has a number of curved grooves **21**, **23**, **25**, which are aligned precisely with the boreholes of the input and output ports.

The rotor **2** is pressed with a contact pressure force against the stator **1**, so that a mutual interface between rotor **2** and stator **1** is implemented, at which the two parts form a seal against one another. The contact pressure force is dimensioned in this case so that the arrangement is still leak-tight even at the highest pressures to be expected.

In the so-called equilibration phase of the system illustrated in FIG. **2**, the switching or injection valve is located in the INJECT position, so that the pump actively flushes solvent through the sample loop in the direction of the column. A required GDV of the entire system or of the automatic sampler **10**, which was determined during the method development or is already predefined, is adjusted by means of positioning of the plunger **53** during the equilibration (GDV of the entire system and additional  $\Delta$ GDV of the metering device **5**).

In the illustrated exemplary embodiment, a position Pos. B is assumed for this purpose, in which the metering device

has a pump volume  $V_B$ . This volume already contains a desired change  $\Delta$ GDV ( $=V_A$ ) of the GDV of the automatic sampler **10** (or of its conventional GDV) and a desired sample quantity  $V_{sample}$  ( $=V_B - V_A$ ) to be received (at a later point in time), wherein, of course, other variants without including a sample volume at such a point in time are also conceivable.

A varied piston positioning during the equilibration and a therefore varied pump volume  $V$  of the volume adjustment device results in a changed pressure and flow (option 1), wherein the volume change can be compensated for in a preferred embodiment of the invention by adapting the flow rate, so that the pressure and flow still remain stable (option 2). The adaptation of the flow rate is performed in this case by activation of the pump **40** by the control unit **60**, which opposes the metering device **5** accordingly.

Subsequently, the valve **3**, as shown in FIG. **3**, switches into a PRESSURE EQUALIZATION position, in which the connecting part **51** and the second connecting part or the supply part **52** of the sample loop have no connection to the other components connected to the injection valve **3** and the sample loop is therefore switched out of the analytical path. The sample loop also has system pressure at this time (high pressure greater than 500 bar or even greater than 1500 bar).

In one variant (only required in the case of a method 1 explained hereafter), the drive **55** of the metering device **5** can move forward briefly, until the plunger **53** moves. In this case, the force on the plunger **53** or the torque of the drive **55** is measured and stored. It is a measure of the pressure in the sample loop (system pressure). In another variant, the pressure is ascertained or monitored by means of a sensor or the plunger position is detected and stored for later use.

Thereafter, the metering device **5** decompresses the sample loop by changing the plunger position from position B to position C or increasing the pump volume from  $V_B$  to  $V_C$  until almost to atmospheric pressure. In this case, the required movement path of the plunger **53** can be ascertained by measuring the force on the plunger **53** or the drive torque during the aspiration of the preceding sample or determined by a pressure sensor in the sample loop.

As shown in FIG. **4**, the switching valve **3** subsequently switches to the WASTE position, in which the quantity of solvent is expelled by the metering device **5**, which corresponds to the sample quantity  $V_{sample}$  ( $=V_C - V_A$ ) to be aspirated.

In the state shown in FIG. **5**, the sample needle **42** was subsequently moved into the sample container **43**, so that a sample volume can be aspirated. For this purpose, the plunger **53** is initially in position A and is controlled by the control unit **60** for the aspiration in position C. In this case, the desired, defined sample volume  $V_{sample}$  ( $=V_C - V_A$ ) is aspirated into the aspirating part **44**, wherein the volume of the sample is preferably less than the volume of the aspirating part **44**, so that no mixing of the sample fluid with the fluid conveyed by the high-pressure pump can occur in the pump volume. FIG. **5** shows the state of the HPLC system after ending the aspiration operation.

In the first LOAD position of the valve **3** shown in FIG. **5**, the grooves **21**, **23**, **25** are aligned with the ports **12** to **16** so that the grooves **23** and **25** connect the two high-pressure ports **14**, **15** or the waste port **12** and the sample loop port **13**. In this LOAD position, the high-pressure pump **40** therefore delivers fluid in the direction toward the chromatography column **41**. Furthermore, the sample loop port **16** is closed pressure-tight.

During the aspirating, the force on the plunger **53** or the drive torque of the metering device **5** can be measured,

wherein the drive torque or the plunger force represents a measure of the atmospheric pressure here and can be used as explained above during the decompression. Of course, it is also conceivable to ascertain, monitor, and/or store for later use the (atmospheric) pressure by means of a pressure sensor.

Subsequently, the switching valve **3** switches to a PRESSURE EQUALIZATION position, in which the connecting part **51** and the second connecting part or the supply part **52** of the sample loop do not have a connection to the other components connected to the injection valve **3**. So as not to interrupt the flow through the chromatography column **41** during the delivery of the volume required for the compression of the sample loop content, the groove **25** in the rotor **2** of the valve is embodied as lengthened accordingly, so that the two high-pressure ports **14**, **15** are also still connected in the PRESSURE EQUALIZATION position.

The sample loop is compressed in this case in one variant (method 1) until the stored (as explained above) drive torque or the plunger force is achieved as a measure of the system pressure. The pressure then again corresponds to the system pressure (high pressure). A compressibility of the sample which deviates from the solvent can cause the resulting plunger position to no longer correspond precisely under certain circumstances to the starting position, position B, of the plunger **53** in this case. In this way, exact precompression of the sample loop to the system pressure is performed with a small negligible deviation of the desired value  $GDV_{target}$  of the GDV.

In another conceivable variant (method 2), the plunger **53**, as shown in FIG. **6**, is navigated to the starting position, position B, again and thus compresses the sample loop. In this case, deviations of the compressibility of the sample in comparison to the compressibility of the solvent result in slight deviations, which are to be neglected, from the system pressure. For this purpose, the achieved GDV corresponds exactly to the desired value  $GDV_{target}$  of the GDV.

To be able to inject the sample volume which is located in the aspirating part **44**, the sample needle **42** is moved into the injection port **45**. This port seals off the needle tip so it is resistant to high pressure. Subsequently, the switching valve or injection valve **3** switches to the inject position, in which the aspirated sample volume is conveyed completely out of the aspirating part **44** to the column **41** (injection) by the solvent conveyed by the pump **40**. If a gradient is navigated for the solvent (chronologically controlled mixing ratio of the solvent) for a chromatography run, no undesired delays advantageously result.

In a preferred embodiment of the invention, a deviation of the GDV caused by the precompression can be readjusted after the injection, wherein the correction movement of the plunger (to position B) is compensated for by corresponding adaptation of the flow rate. In this way, with exact precompression, an exact (adjusted or desired)  $GDV_{target}$  can nonetheless be obtained. In addition, it is also possible (in particular if the sample volume after the compression is greater than the desired  $GDV_{target}$ ) to readjust the desired  $GDV_{target}$  during the chromatography run.

Although it is described differently in the exemplary embodiment, however, it is, of course, also conceivable to set or vary the GDV (deviating from a system-specific  $GDV_{actual}$ ) to a desired amount  $GDV_{target}$  also during a chromatography run instead of before. For this purpose, the pump **40** can be activated in opposition to the change of the metering device **5** accordingly via the control unit **60**, to keep the flow rate or the volume stream to the column **41** constant.

Optionally, at the end of the chromatography run, the deviation  $\Delta GDV (=GDV_{target} - GDV_{actual})$  and therefore GDV or the mixing volume can be set to a minimum (minimal pump volume) by setting the plunger **53** at position 0, to shorten the washing-out time of the system, in particular of the sample loop.

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List of reference signs

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1	stator
2	rotor
3	injection valve
5	metering device
10	automatic sampler or chromatography system
12	waste port
13	second sample loop port
14	further high-pressure port
15	high-pressure port
16	first sample loop port
21, 23, 25	grooves
40	high-pressure pump
41	separating column
42	sample needle
43	sample container
44	aspirating part
45	injection port
47	waste line
50	syringe
51	first connecting part
52	supply part
53	plunger
55	drive
60	control unit
V	pump volume
VA	pump volume in position A
VB	pump volume in position B
Vc	pump volume in end position C
$V_{sample}$	sample quantity
$V_{max}$	maximum volume of the metering device
Pos. 0	lowermost position of the plunger
Pos. A	position A of the plunger
Pos. B	position B of the plunger
Pos. C	end position of the plunger
GDV	gradient delay volume of the entire system
$GDV_{actual}$	system-specific value of the GDV
$GDV_{target}$	desired value of the GDV
$\Delta GDV$	deviation of the GDV due to corresponding plunger position

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What is claimed is:

1. A method for setting a gradient delay volume of a liquid chromatography system for a chromatography run, the method comprising:

setting, and inputting into a control unit of the liquid chromatography system, a target gradient delay volume ( $GDV_{target}$ ), wherein the target gradient delay volume ( $GDV_{target}$ ) is predefined based on an earlier chromatography run of the liquid chromatography system;

calculating, by the control unit, a difference of the gradient delay volume ( $\Delta GDV$ ) between the target gradient delay volume ( $GDV_{target}$ ) and an actual gradient delay volume ( $GDV_{actual}$ ) of the liquid chromatography system for the chromatography run;

controlling, by the control unit, a metering device of the liquid chromatography system, the metering device includes a syringe and a plunger, the plunger is configured to be guided in a pressure-tight and displaceable manner, wherein controlling the metering device is accomplished by moving the plunger within the syringe, the metering device further including a variable pump volume V defined by the plunger, to adjust the pump volume V by adjusting a position of the

## 11

plunger within the syringe, in which the adjusted volume is equal to the calculated difference of the gradient delay volume; and

performing the chromatography run on the liquid chromatography system using the adjusted volume and a solvent gradient, the liquid chromatography system further comprising an injection valve, a pump connected to the injection valve, a separation column connected to the injection valve, and a sample loop extending between a pair of sample loop ports of the injection valve, wherein the sample loop comprises the pump volume V of the metering device.

2. The method of claim 1, in which the difference of the gradient delay volume is calculated with an equation, the equation comprising:

$$\Delta\text{GDV}=\text{GDV}_{\text{target}}-\text{GDV}_{\text{actual}}, \text{ where}$$

$\Delta\text{GDV}$  is the difference of the gradient delay volume due to the position of the plunger within the metering device,

$\text{GDV}_{\text{target}}$  is the target gradient delay volume, and  $\text{GDV}_{\text{actual}}$  is the gradient delay volume of the liquid chromatography system.

3. The method of claim 1, in which the liquid chromatography system includes an injection port selectively connected to the separation column via fluidly interconnected components, in which the actual gradient delay volume comprises a holding capacity of the fluidly interconnected components extending from the metering device to the separation column.

4. The method of claim 1, in which the difference of the gradient delay volume is less than or equal to 1000 microliters.

5. The method of claim 1, in which the performing of the chromatography run step includes injecting the sample into

## 12

the separation column, in which the setting of the target gradient delay volume occurs after the sample injection.

6. The method of claim 5, in which the controlling the metering device step includes adjusting the plunger of the pump to compensate for the adjusted volume V of the metering device so that a flow rate and a pressure of the sample entering the separation column is essentially constant.

7. The method of claim 1, in which the performing of the chromatography run step includes injecting the sample into the separation column, in which the setting of the target gradient delay volume occurs before the sample injection.

8. The method of claim 1, wherein the performing of the chromatography run step includes, at the end of the chromatography run, adjusting the position of the plunger in the metering device so that the volume of the metering device is zero.

9. The method of claim 1, in which the performing of the chromatography run step includes taking a sample into the sample loop with the metering device.

10. The method of claim 1, in which the sample loop further comprises:

a first connecting part and a second connecting part, in which the first connecting part is connected to a first sample loop port of the pair of sample loop ports and to the metering device,

in which the second connecting part is connected to a second sample loop port of the pair of sample loop ports and to the metering device,

in which the second connecting part comprises an aspirating part and a supply part, in which the aspirating part and the supply part are configured to be separated, in which the aspirating part is connected to the metering device and the supply part is connected to the injection valve.

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